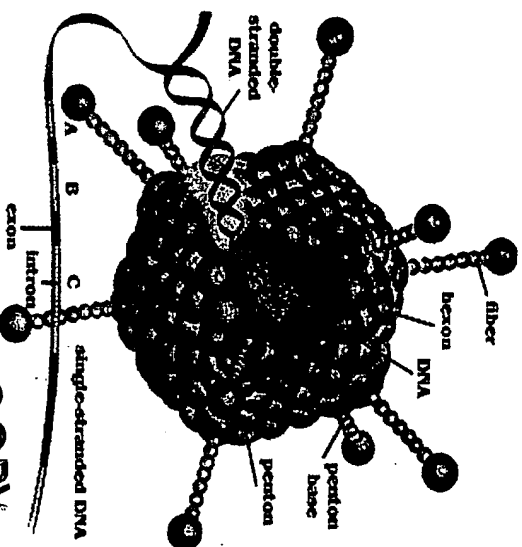


• Crucell



Patent application 09/348,354 “Chimeric adenoviruses



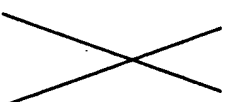
BEST AVAILABLE COPY

Modified vector library

Adenovirus type 5

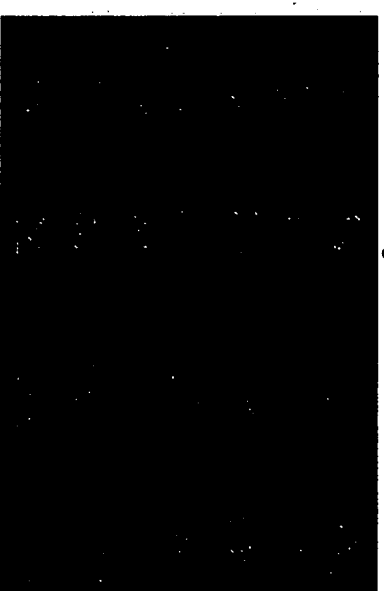
Therapeutic

FIBER



Library of Fibers

PCR amplify



Production on PER.C6



Adenovirus type 5
1:100

Definition of "Tropism"

US6,127,525:

specificity or natural affinity for certain tissues
of an organism".

Definition is limited to binding of virus to cell type due to
compatibility of virus and cellular receptor.

Crucell:

The sum of biological processes that determine whether
a virus can transfer a gene in vivo to a particular cell,
organ or tissue.

- # Anatomical barriers
- # Viral lysis by serum components (non-antibody related)
- # Neutralizing antibodies
- # Receptor-virus compatibility
- # Vector stability

Model: Anatomical barriers

Due to for instance vessel wall barrier the virus is not able to reach the target of interest.

Due to the size of Adenovirus, penetration in tumor tissue is severely limited.

3) Expression profile of Ad5 receptor does not correlate with observed Ad5 infection patterns in rodents

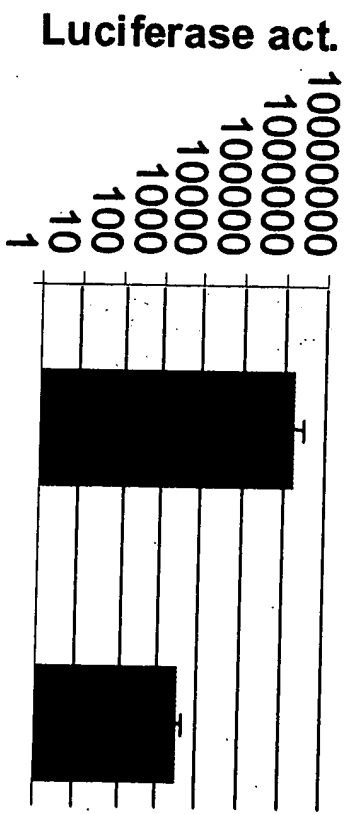
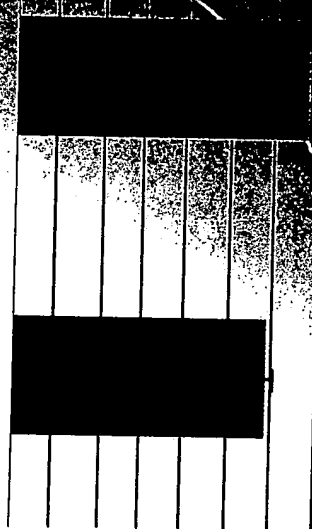
(Fechner *et al* Gene Ther. 1999 Sep;6(9):1520-35)

Cell: viral lysis by serum

Ad5.Fib16 monkey (pre-treatment)

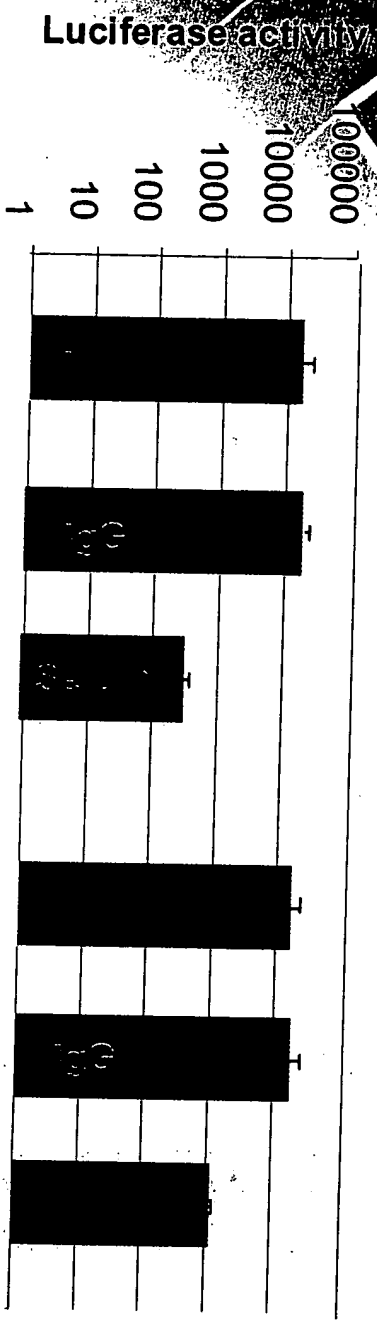
Ad5

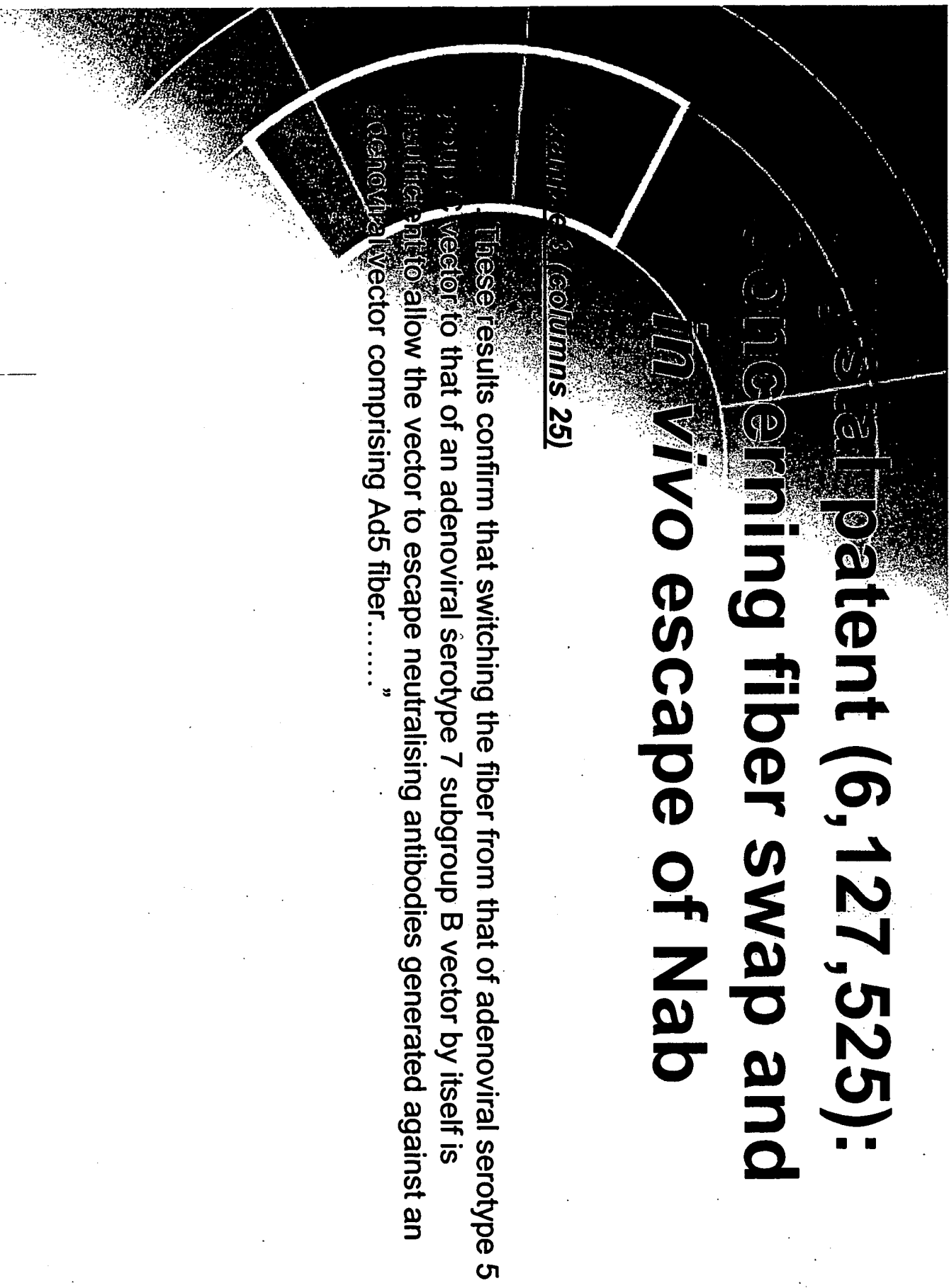
Ad5.Fib16



1/4 diluted serum, dose: 500 vp/ cell, A549 cells

Serum effect is independent of antibodies



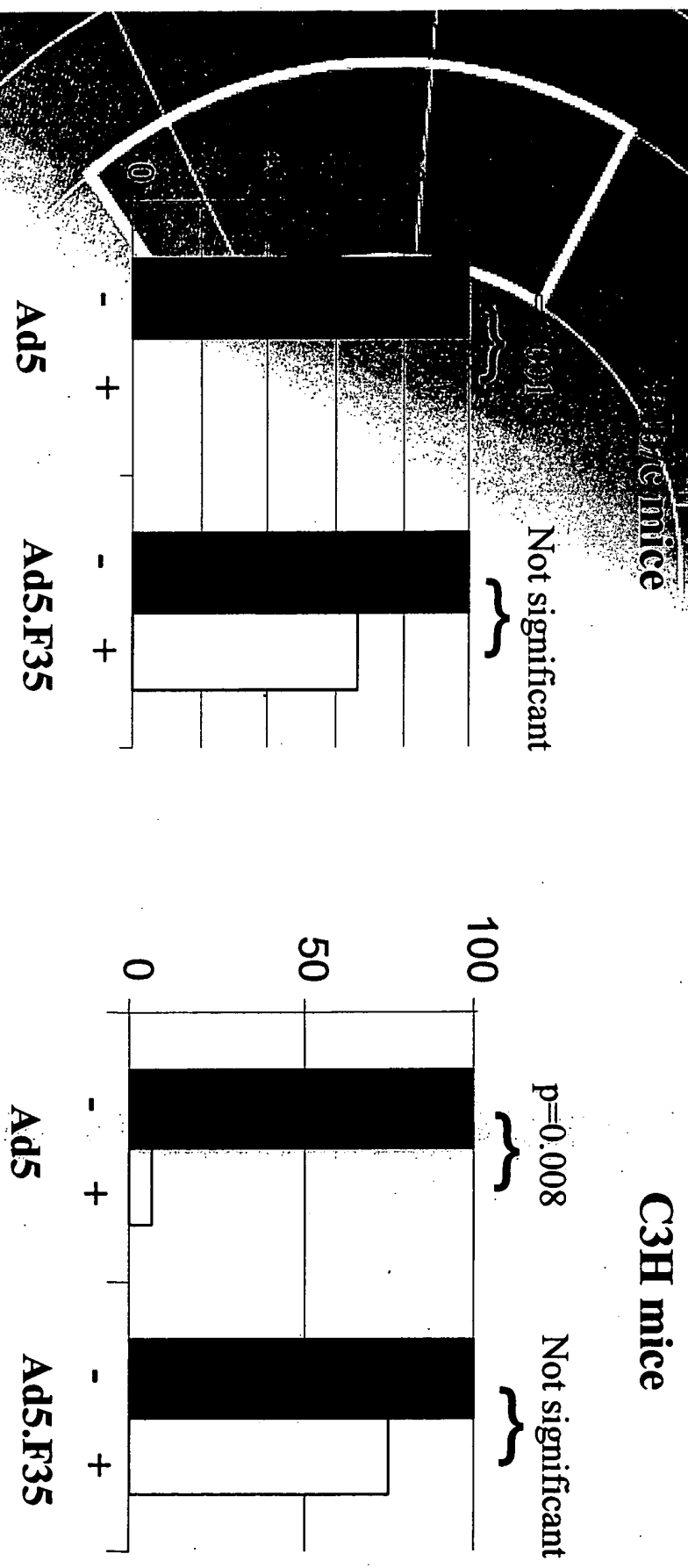


State patent (6,127,525): concerning fiber swap and *in vivo* escape of Nab

Example 3 (columns 25)

These results confirm that switching the fiber from that of adenoviral serotype 5 group 1 vector to that of an adenoviral serotype 7 subgroup B vector by itself is sufficient to allow the vector to escape neutralising antibodies generated against an adenoviral vector comprising Ad5 fiber.....”

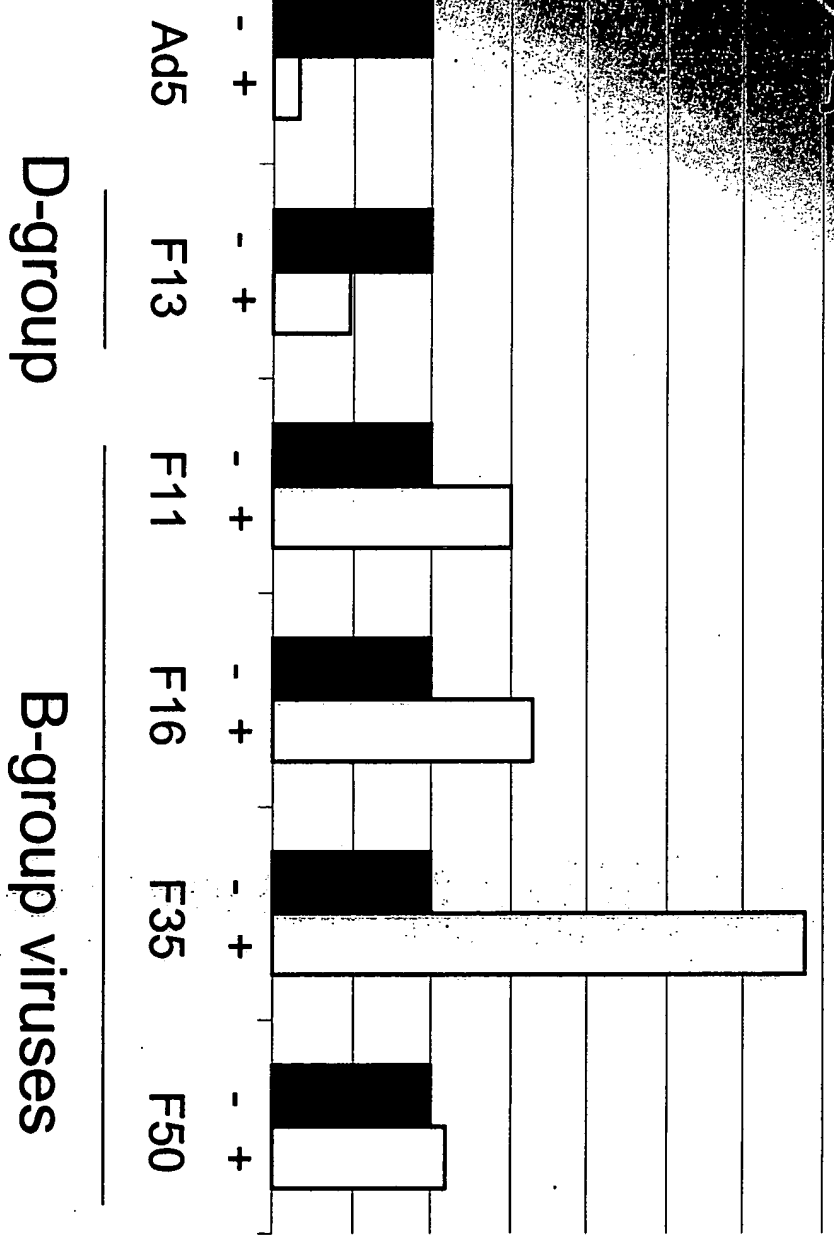
Anti-Neutralizing antibodies



P values determined via "Mann-Whitney U" test

Neutralizing antibodies

C57/ B16 mice



P values determined via "Mann Whitney U test"

Advances in structural design of Fiber-chimeric vector

Patent:

Complete deletion of Ad5 fiber and insertion of complete Ad7 fiber.

et al., J. Virol Vol 70, p2120:

"We have shown that amino acid homology between the tail regions of Ad5 and Ad7 is sufficient to allow functional replacement of the Ad5 fiber with Ad7 fiber..."

Crucell:

~ Retained Fiber tail of Ad5 to ensure proper interaction with Ad5 penton-base (i.e. homology between Ad7 and Ad5 in fiber tail region is 57% on a.a. level)

Substantial difference in vector stability expected